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14. ABSTRACT

We have demonstrated that a low band gap D-A conjugated polymer **P3**, that absorbs in the NIR (~800 nm), can be fabricated into spherical nanoparticles (**nano-P3**) using Pluronic F127 as a soft template. **Nano-P3** (~67 nm) was found to be very stable in aqueous media. A heating efficiency curve showed that it took ~7 µg of **nano-P3** to change the temperature of the solution by 20^{o}C after one minute. This number is at a lower concentration than other donor acceptor polymer nanoparticles, such as nano-PCPDTBSe and nano-PCPDTBT, which may be due to **P3** having a 20% greater molar absorptivity than PCPDTBSe. The nanoparticles, in the absence of NIR light, showed no significant toxicity towards CT26 colorectal cancer cells at concentrations of 5-200 µg/mL. Cell viability assays showed that in the presence of NIR light, **nano-P3** was shown to generate significant heating to destroy colorectal cancer cells at very low nanoparticle conentration (~ 15 µg/mL). Using the lowest possible concentration for photothermal ablation is advantageous for translating D-A ECPNs to clinical applications.

15. SUBJECT TERMS :nothing listed

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1. INTRODUCTION:

CRC is the third most commonly diagnosed cancer and will contribute to ~150,000 new cases this year with one-third of those cases leading to death from metastatic disease.[1] CRC becomes increasingly difficult to treat once it has metastasized to other areas of the abdomen.[2] mCRC of the peritoneum is widely regarded as a terminal condition in clinical studies, with survival rates on the order of less than six months.[3] Treatment options for mCRC are lacking. Systemic chemotherapy is not effective against peritoneal carcinamatosis because the drug cannot pass the peritoneal-plasma barrier. Recently, a new treatment frequently used by our institution, Heated intraperitoneal chemoperfusion (HIPEC), has increased the median survival rate of patients with advanced stage colorectal cancer from 0.7-6 months to 13-60 months. Although mCRC survival rates have improved using HIPEC, some of the drawbacks to the procedure include long operating times and higher doses of chemotherapy drug (2L) compared to conventional systemic treatments.[4]

Near-infrared (NIR) photothermal therapy using nanomaterials has gained much attention as a minimally invasive and efficient treatment for cancer.[5] Body tissue is transparent in the NIR window from 700-1100 nm, making NIR induced photothermal therapy a very attractive option for cancer therapeutics. Upon stimulation from NIR light, excitation of vibrational resonances in the nanomaterial allow for heat generation which can destroy the surrounding cancer cells. Electrically conducting polymers (ECPs), like polyaniline, polypyrrole and poly(ethylenedioxythiophene) (PEDOT), have been shown to photothermally ablate cancer cells under NIR stimulation, while still resulting in low toxicity to eukaryotic cells.[6,7,8]Currently, ECPs have 1) relatively low absorbance in the NIR and 2) are not cancer cell selective. Low band gap donor acceptor (D-A) ECPs are copolymers that incorporate both an electron donating monomer and an electron accepting monomer into their backbone. Through judicious choice of co-monomers used, D-A ECPs can be tuned to have excellent absorption properties in the NIR window from 700-900 nm. Our group has shown that D-A electrically conducting polymer nanoparticles (ECPNs) are not cytotoxic and can photothermally ablate CRC cells under NIR stimulation *in-vitro*.[9] Through chemical modification, these D-A ECPNs can be fitted with functional groups that will allow for targeting of the ECPNs to the cancer cell surface. The overall objective of the proposed research is to synthesize D-A ECPNs that absorb between 700-900 nm to target and treat mCRC of the abdomen. This could translate into clinical application using an open abdomen procedure similar to HIPEC.

2. KEYWORDS: colorectal cancer; photothermal therapy; conjugated polymer; metastases; HIPEC

3. OVERALL PROJECT SUMMARY:

Current Objectives:

For the first year of the DoD cancer visionary postdoctoral fellowship, my goal was to complete **Task 1** in my statement of work and the four subtasks included within it. **Task 1** involves the synthesis and characterization of the two novel Donor-Acceptor (D-A) polymers **P3**-**P4** that will be used for this study. Below you will find **Task 1** with **Subtasks 1**-**4** from my statement of work.

Task 1: Synthesis and Characterization of Donor-Acceptor (D-A) Polymers **P3**-**P4** (timeframe, months 1-12)

Subtask 1: 4,4-*bis*(2-ethylhexyl)-2,6-*bis*(trimethyl-stannyl)-4H-cyclopenta-[2,1-b;3,4-b']dithiophene donor monomer (**M1**), a 3,6-dithien-2-yl-2,5-di(2-ethylhexyl)-pyrrolo[3,4-c]pyrrole-1,4-dione acceptor

monomer (**A**) will be synthesized (timeframe months, 1-6). We will employ a Stille coupling polymerization of 4,4-*bis*(2-ethylhexyl)-2,6-*bis*(trimethyl-stannyl)-4H-cyclopenta-[2,1-b;3,4-b']dithiophene with 3,6-dithien-2-yl-2,5-di(2-ethylhexyl)-pyrrolo[3,4-c]pyrrole-1,4-dione to form polymer **P3**.

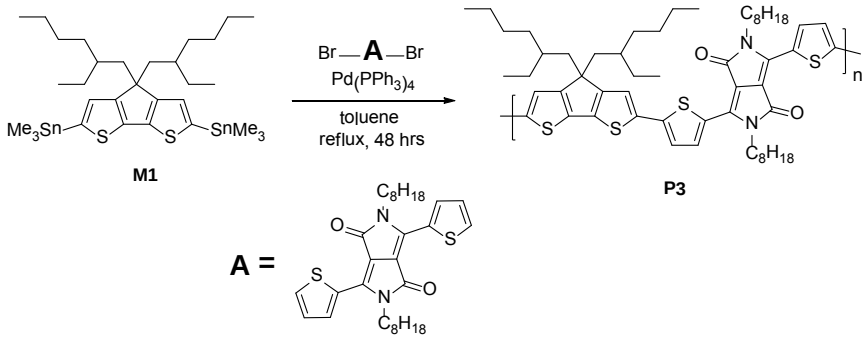
Subtask 2: Full Characterization of **M1**, **A** and D-A Polymer **P3** will be undertaken (timeframe, months 3-8) Characterization methods for monomers and polymer Include Electrospray Ionization Mass Spectrometry (ESI-MS), Gas Chromatography Mass Spectrometery (GC-MS), Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR), Carbon Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMR), Tin Nuclear Magnetic Resonance Spectroscopy (^{119}Sn-NMR), Fourier Transform Infrared Spectroscopy (FT-IR), Absorbance Spectroscopy (UV-Vis) and Thermogravimetric Analysis (TGA).

Subtask 3: Synthesis of Folic acid functionalized acceptor Monomer **A2** and Folic acid functionalized D-A Polymer **P4** (timeframe, months 7-10). A similar synthesis to Subtask 1 will be completed. Through attachment of folic acid to the polymer backbone, we will be able to target the D-A polymer to the cancer cell surface.

Subtask 4: Full Characterization of **A2** Monomer and D-A Polymer **P4** (timeframe, months 8-12) utilizing the same techniques as above.

Results, Progress and Accomplishments

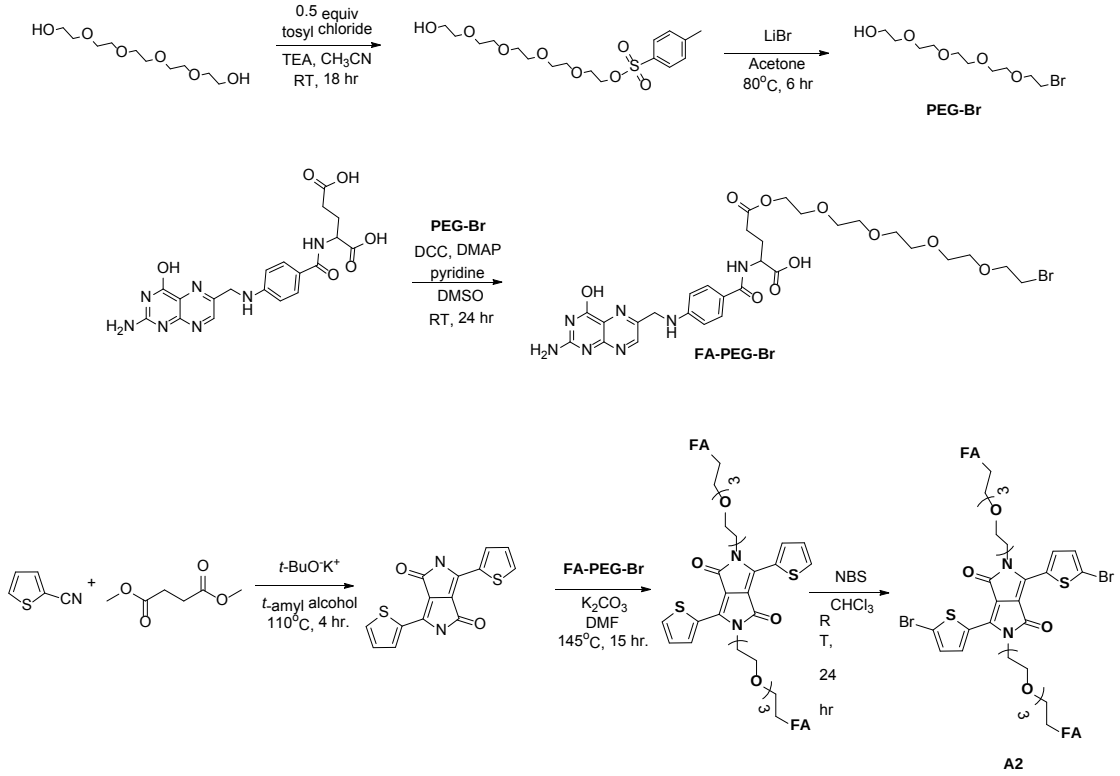
We were able to complete **Subtask 1** and **Subtask 2**, which involves the synthesis and characterization of **M1**, **A** and **P3** (Figure 1, see appendix for experimentals and full characterization).



Scheme 1: Palladium catalyzed Stille coupling polymerization of **P3**

We have started on **Subtask 3** and **Subtask 4** and have completed close to half. We ran into difficulties in the synthesis of the folic acid functionalized polymer **P4**. Firstly, in the fellowship application, we wanted to attach folic acid to the DPP monomer backbone through a 6-bromo-1-hexanamine linker. After further discussion with mentors and colleagues, we determined that the 6-bromo-1-hexanamine linker is too hydrophobic and if we wanted the folic acid moieties on the surface of the nanoparticle to be pointing out toward the water, we needed to use a more hydrophilic linker. The new linker we chose was a substituted tetraethyleneglycol. The complete

synthesis of the folic acid functionalized monomer can be seen in **Scheme 2** and the experimentals can be found in the appendix.



Scheme 2: Synthesis of monomer **A2**.

Secondly, we were able to successfully synthesize **FA-PEG-Br** from **Scheme 2** above. During the second half of synthesis, we couple **FA-PEG-Br** to the DPP monomer to give **A2**. We are still working out the reaction conditions needed to successfully complete this synthesis. The **FA-PEG-Br** compound is only partially soluble in DMF, which is the solvent needed for this coupling so the reaction didn't work. We will be trying other aprotic solvents such as DMA, DMSO or NMP to in order to fully solubilize the **FA-PEG-Br**.

Since we have not been able to fully complete **Subtask 3** and **Subtask 4** of **Task 1** because experiments are still on-going, we began some of the experiments outlined in **Task 2**, which is supposed to be started in Year 2 of the fellowship. **Task 2** is outlined below.

Task 2: Polymer Nanoparticle Formulation and Characterization (timeframe, months 12-20)

Subtask 1: Synthesis of D-A ECPN ***nano*-P3** and folic acid functionalized D-A ECPN ***nano*-P4** (timeframe, months 12-14) will be set up by a phase separation and sonication method previously developed in our lab to give stable nanoparticle suspensions.

Subtask 2: Characterization of D-A ECPNs ***nano*-P3** and ***nano*-P4** (timeframe, months 13-16) will be completed using dynamic light scattering (DLS) to judge the nanoparticle size. We will also you zeta potential measurements to determine the surface charge of the particles.

Transmission electron microscopy (TEM) will allow be utilized to help us determine the size and shape of the nanoparticles to compare to the DLS measurements.

Subtask 3: Heating Studies of D-A ECPNs ***nano*-P3** and ***nano*-P4** (timeframe, months 16-18) will be done. An 800 nm laser diode will irradiate solutions and generate heat. The solutions will contain different concentrations (5 ug/mL to 1000 ug/mL) of nanoparticles. The power density will be varied (100mW – 2W) as well as the amount of time the nanoparticles are irradiated with the 808 nm laser (30s – 10 min) in order to determine the most efficient combination for treatment. The solution temperature will be measured using a thermocouple.

Subtask 4: Cell Ablation and Cytotoxicity Studies of D-A ECPNs ***nano*-P3** and ***nano*-P4** with RKO, HCT116 and CT-26 Colorectal Cancer Cells (timeframe, months 18-24) will be undertaken. Data from **Subtask 3** will allow us to determine optimum conditions needed to destroy colorectal cancer cells *in-vitro*.

We were able to complete **Subtasks 1-3** of **Task 2** and part of **Subtask 4** for the control polymer D-A ECPN ***nano*-P3**. The results of **Subtasks 1-4** can be seen below.

Synthesis of D-A ECPN *nano*-P3

P3 was synthesized using a Stille coupling procedure as mentioned above. The polymer was extracted with methanol and hexanes to remove low molecular weight materials. Finally, the polymer was extracted with chloroform to give high molecular weight **P3**. The molecular weight (M_n) of **P3** was determined to be 21425 by gel permeation chromatography (GPC). UV-visible spectrum of **P3** in chloroform can be seen in **Figure 1**. The absorbance maximum at 760 nm is due to the charge transfer between the cyclopentadithiophene donor and the diketopyrrolopyrrole acceptor. The optical band gap can be predicted from the absorbance onset (λ_{onset} = 954 nm) of **P3**. From this, we can calculate a band gap of 1.32 eV for **P3**. **P3** was formed into D-A electrically conducting polymer nanoparticles (ECPNs) (***nano*-P3**) using a nano-precipitation method. **P3** was dissolved in THF and rapidly injected into a water solution containing pluronic F127. Pluronic F127 is a biocompatible, non-ionic surfactant that is FDA approved for use as a drug delivery vehicle.[10,11,12] Pluronic F127 was used as a soft template to shape the polymer chains into aqueous-stable spherical nanoparticles.[13] Upon injection, the **P3** will self-assemble within the hydrophobic core of the spherical micelle. The PEO chains of Pluronic F127 will point out towards the water layer to provide **nano-P3** with aqueous stability. Through use of transmission electron microscopy, we confirmed that **the nano-P3** had an average diameter ranging from 20-100 nm. The polydispersity can be decreased through centrifugation to remove larger nanoparticles and aggregates. After centrifugation, dynamic light scattering (DLS) experiments confirmed that the hydrodynamic diameter of ***nano*-P3** was 67 nm (PDI = 0.5). Zeta potential measurements determined that the surface charge of ***nano*-P3** was -31.8.

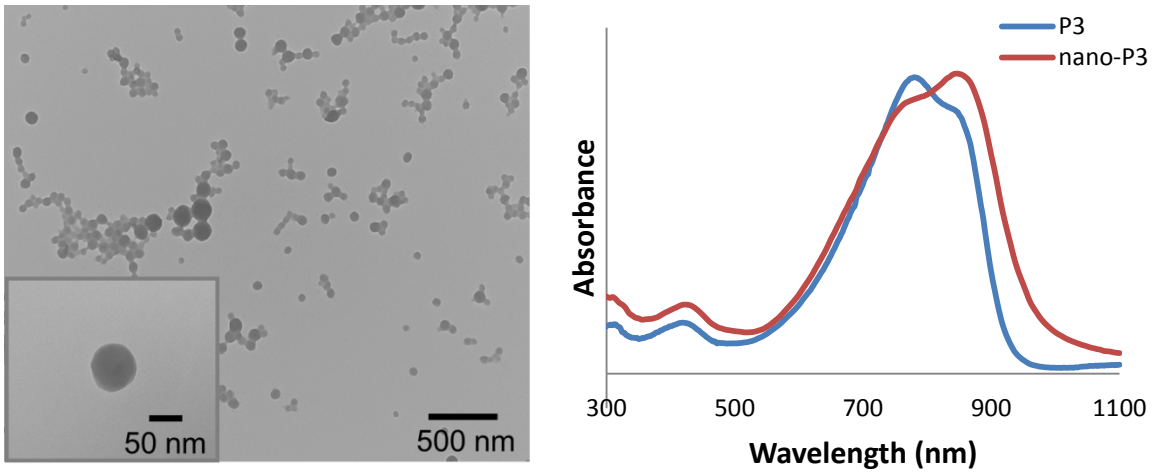


Figure 1: TEM images of ***nano-P3*** drop cast from water. UV-visible spectrum of polymer **P3** in CHCl3 (blue) and ***nano*-P3** in water (red)**.**

The photothermal efficacy of ***nano*-P3** was tested and compared to a material that is known to heat very well under NIR stimulation. Separate concentrations of aqueous-stable nano-P3 was illuminated with an 800 nm (3W, 0.5 cm diameter spot size) diode laser for one minute. After laser treatment, a thermocouple was used to measure the solution temperature. The change in temperature vs. concentration for ***nano*-P3** is shown in Figure 2. It took ~7 µg of **nano-P3** to change the temperature of the solution by 20°C after one minute. The donor acceptor polymer ***nano*-P3** was shown to heat better at lower concentration than other donor acceptor polymer nanoparticles, such as nano-PCPDTBSe and nano-PCPDTBT. This could be due to **P3** having a 20% greater molar absorptivity than PCPDTBSe.[14,15]

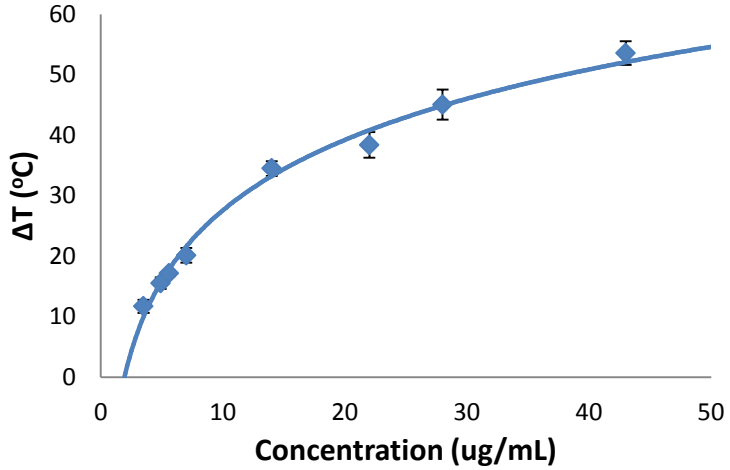


Figure 2: Photothermal efficiency plot of the change in temperature (ΔT) of ***nano*-P3** at a range of concentrations in water.

In order to determine whether the ***nano*-P3** was harmful to cells, an *in-vitro* cytotoxicity assay was performed in the absence of NIR light. The results of the cytotoxicty assay can be seen in Figure 3. ***nano*-P3** had no significant toxic effect to CT26 colorectal cancer cells at any concentrations up to 200 ug/mL. Cell ablation studies were performed by irradiating the CT26 colorectal cancer cell solutions with an 800 nm (either 3W or 1W, 0.5 cm spot size) diode laser for 1 minute. The results of the cell

ablation studies are shown in Figure 3. For 1W laser irradiation, there was little cell kill up to 40 µg/mL; however, cell viability decreased to less than 20% for concentrations above 100 µg/mL. For 3W laser irradiation, cell kill was almost immediate (7.5 ug/mL), however cell viability didn't decrease to fewer than 20% until concentrations were above 15 ug/mL. These results illustrate that ***nano*-P3** has enhanced photothermal efficiency (7X greater) at 3W compared to 1W.

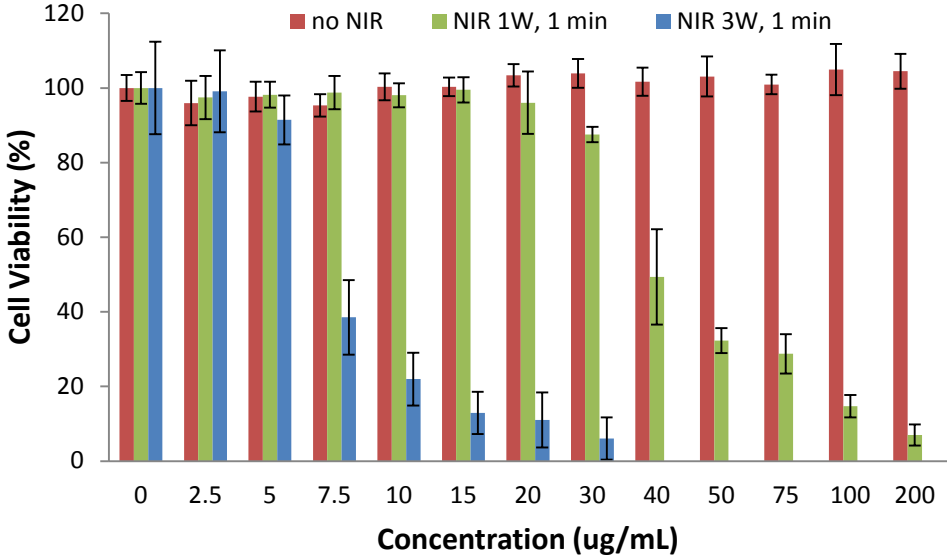


Figure 3: Cell Viability of CT26 cells for a range of concentrations of **nano-P3** under no NIR stimulation (red), NIR stimulation (1W, 1 min, green) and NIR stimulation (3W, 1 min, blue).

4. **KEY RESEARCH ACCOMPLISHMENTS:** Nothing to report

5. **CONCLUSION:** Summarize the importance and/or implications with respect to medical and /or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.

In conclusion, we have demonstrated that a low band gap D-A conjugated polymer P3, that absorbs in the NIR (~800 nm), can be fabricated into spherical nanoparticles (nano-P3) using Pluronic F127 as a soft template. **Nano-P3** (~67 nm) was found to be very stable in aqueous media. A heating efficiency curve showed that it took ~7 µg of **nano-P3** to change the temperature of the solution by 20^{o}C after one minute. This number is at a lower concentration than other donor acceptor polymer nanoparticles, such as nano-PCPDTBSe and nano-PCPDTBT, which may be due to **P3** having a 20% greater molar absorptivity than PCPDTBSe. The nanoparticles, in the absence of NIR light, showed no significant toxicity towards CT26 colorectal cancer cells at concentrations of 5-200 µg/mL. Cell viability assays showed that in the presence of NIR light, **nano-P3** was shown to generate significant heating to destroy colorectal cancer cells at very low nanoparticle conentration (~ 15 µg/mL). Using the lowest possible concentration for photothermal ablation is advantageous for translating D-A ECPNs to clinical applications. Future goals would be to complete **Tasks 1** and **2** outlined in the SOW and start the mouse model of mCRC to determine how well these nanoparticles could treat mCRC *in vivo*. This is important because CRC is the fourth most common cancer among military personnel behind testicular, prostate and breast cancer. Early screening for CRC is down among military personnel. Only 58% of

military men and women who should be screened for CRC have been screened. The risk of developing cancer among military personnel may be different than the general population due to differences in amount of exercise, smoking, diet and alcohol consumption. CRC becomes increasingly difficult to treat once it has metastasized to other areas of the abdomen. Synthesizing new polymer-based nanomaterials that can selectively target and treat CRC will have an immense impact on the military's and the general public's health. The goal is to shorten current treatment times compared to conventional methods. Our group will localize hyperthermia to where it is needed in the abdomen by targeting the polymeric nanoparticles to the cancer cells using folic acid.

6. **PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:** Nothing to report

7. **INVENTIONS, PATENTS AND LICENSES:** Nothing to report

8. **REPORTABLE OUTCOMES:**

The work supported by this award has allowed to me to increase my knowledge in the area of polymer chemistry. Recently, I received a senior scientist position in applied research at L'Oreal USA in New Jersey. The company was impressed with my polymer chemistry knowledge and background as well as my knowledge of many different characterization methods in polymer synthesis. This fellowship has allowed me to gain some greater experience in polymer science. The company was particularly impressed with my biomedical background and chemical knowledge that was a result of the some of the work contained within this fellowship.

9. **OTHER ACHIEVEMENTS:** Nothing to report

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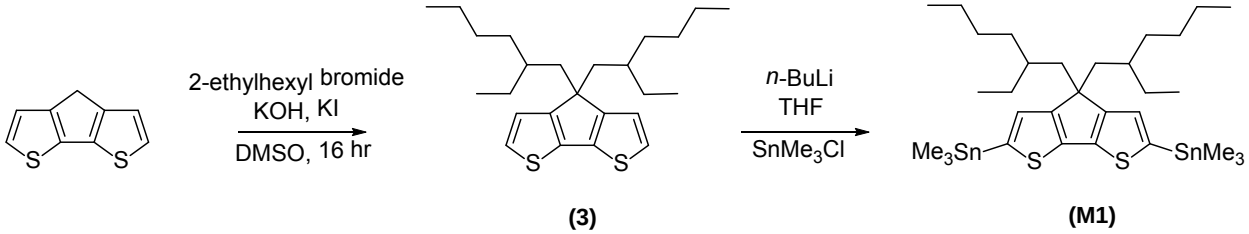
11. **APPENDICES:**

Synthesis of 4,4-Bis(2-ethylhexyl)-4*H*-cyclopenta[2,1-*b*:3,4-*b'*]dithiophene (3). To a round bottom flask was added 4*H*-Cyclopenta-[2,1-*b*:3,4-*b'*]dithiophene (3.41 g, 0.02 mol) and DMSO (115 mL) under nitrogen gas was added sodium iodide (115 mg, 0.08 mmol) and 2-ethylhexyl bromide (7.4 g, 0.04 mol) followed by ground KOH (4.3 g, 0.076 mol). The reaction was stirred overnight. Water was added, and the reaction was extracted with ethyl ether. The organic layer was dried over magnesium sulfate and evaporated. The oil was purified by flash chromatography using hexanes as eluent. The product was obtained as a colorless oil. Yieild 6.6 g, 82%). ^{1}H-NMR was comparable to the literature values.[1]

Synthesis of 4,4-*Bis*(2-ethylhexyl)-2,6-*bis*(trimethylstannyl)-4H-cyclopenta-[2,1-b;3,4-b']-dithiophene (M1). 4,4-bis(2-ethylhexyl)-4*H*-cyclopenta[2,1-*b*:3,4-*b¢*]dithiophene (6.6 g, 0.016 mol) was dissolved in dry THF (130 mL). The solution was cooled to -78°C for 30 min, and 1.6M n-butyllithium (in hexanes, 41.2 mL) was added dropwise. The reaction was stirred at this temperature for 1 h and allowed to warm to room temperature over 1 hr. The reaction was cooled back down to -78 °C, and trimethyltin chloride (1 M in hexanes, 65 mL) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight. Water was added to the solution and the solution was extracted with ethyl ether. The organic layer was separated and dried over magnesium sulfate and then evaporated. The bis(trimethyltin) monomer was obtained as a tan viscous oil. Yield (10.8 g, 91%). ^{1}H-NMR was comparable to the literature values.[1]



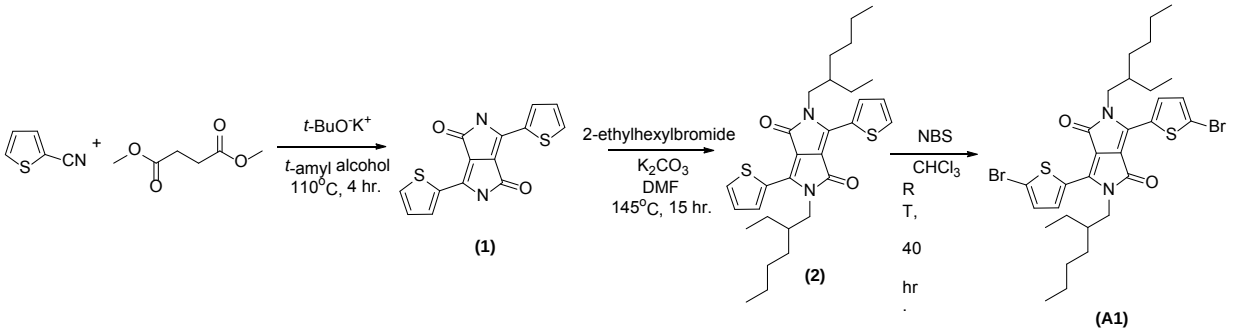
Scheme 1: The synthesis of 4,4-*Bis*(2-ethylhexyl)-2,6-*bis*(trimethylstannyl)-4H-cyclopenta-[2,1-b;3,4-b']-dithiophene (M1).

Synthesis of 2,5-Diethylhexyl-3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (A). The following procedure is similar to previously published methods (Scheme 1).[2]

*3,6-Dithiophen-2-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (**1**)*. Potassium *tert*-butylate (30 g, 0.27 mol) was added to a 3 neck round bottom flask containing t-Amyl alcohol (160 mL) under nitrogen. The mixture was heated to 110oC for 1 hour and 2-thiophenecarbonitrile (19 mL, 0.20 mol) was added dropwise. The mixture was stirred for 30 min and then a solution of dimethyl succinate (16 mL, 0.013 mol) in *t*-amyl alcohol (30 mL) was added dropwise over 1 hr and allowed to stir for 1 hr. The mixture was heated for another 2 hr. The mixture was cooled to 50oC, diluted with methanol (150 mL) and water (50 mL). The mixture was brought back to 110oC for 1 hr. After cooling to RT, the mixture was poured over 500 g of ice and neutralized with conc. hydrochloric acid (100 mL) and 250 mL of MeOH was added. The mixture was stirred for 30 min. The mixture was vacuum filtered and the solid was added to water and filtered a second time. The filtrate was rotovapped and fresh MeOH was added to precipitate more product. The second precipitation was filtered and both of the precipitates were combined and allowed to dry in air to give a final product as a purple powder (16.3 g, 44%). ^{1}H-NMR was comparable to the literature values.[2]

*2,5-Diethylhexyl-3,6-dithiophen-2-ylpyrrolo[3,4-c]pyrrole-1,4-dione (**2**)*. Compound 1 (8.72 g, 0.029 mol) was added to DMF (135 mL) in a 3 neck round bottom flask. The mixture began to heat and anhydrous potassium carbonate (16.5 g, 0.12 mol) was added to the mixture. The mixture was stirred at 135oC under nitrogen for 1 hr. 2-Ethylhexylbromide (25 mL, 0.14) was added drop-wise and the reaction was stirred for an additional 15 hr at 135oC. The solution was cooled to room temperature, poured into 600 mL of ice water, and stirred for 1 hr. The product was vacuum filtered to give a pastey purple solid. The solid was stirred in 250 mL of methanol to dissolve impurities and vacuum filtered again to give a red solid (3.62 g, 30%). ESI-MS (*m/z*) calc'd for C30H40N2O2S2 [M]+: 525.25; found: 525.26. ^{1}H-NMR was comparable to the literature values.[2]

2,5-Diethylhexyl-3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (**A1**). Compound 2 (3.62 g, 7 mmol) and N-bromosuccinimide (2.81 g, 15 mmol) was dissolved in chloroform (150 mL) in a two-neck round bottom flask under argon. The solution was protected from light and stirred at RT for 24 hr. After 24 hr, the mixture was poured into methanol, vacuum filtered, washed with hot methanol twice and dried under vaccuum. Yield (2.95 g, 62%). ^{1}H-NMR was comparable to the literature values.[2]



Scheme 2: The synthesis of 2,5-Diethylhexyl-3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (A1)

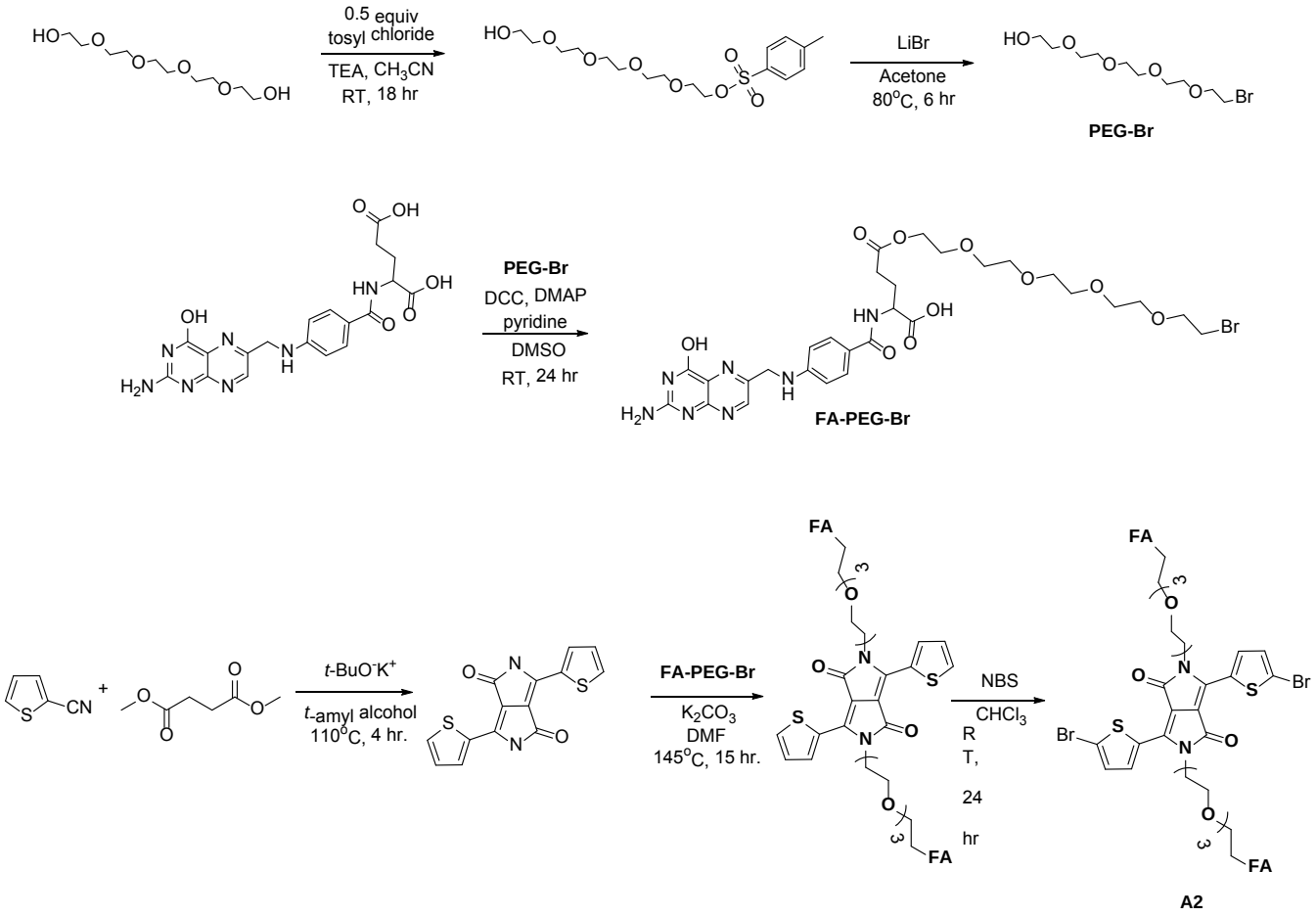
Synthesis of Folic Acid functionalized 3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (A2). The following procedure is similar to previously published methods (Scheme 2) [3-4]

Synthesis of **12-(*p*-Tolylsulfonyl)-3,6,9,12-tetraoxadodecan-1-ol.** Tetraethyleneglycol (10 g, 0.05 mol) was added to acetonitrile (150 mL) in a 3 neck round bottom flask equipped with two stoppers and a dropping funnel. Triethylamine (7 mL) was added and the mixture was stirred for 15 min. Toluene-*p*-sulfonylchloride (10 g, 0.05 mol) was dissolved in acetonitrile (50 mL) and added dropwise to the solution over 1h. The mixture was stirred for an additional 20 h at RT. The white triethylamine hydrochloride precipitate was filtered off and washed with acetonitrile. The solution was concentrated in vacuo and the residue was added to methylene chloride and extracted 2X with water. The organic phase was dried over MgSO4 and evacuated to give a yellow oil. The oil was purified via column chromatography using silica gel and chloroform-acetone (10:3) eluent to yield a yellow oil (12 g, 73%). ^{1}H-NMR (300 MHz; CHCl3)**:** 7.73 (d, 2H aromatic), 7.29 (d, 2H, aromatic), 4.19 (m, 2H, CH2OH), 3.64-3.53 (m, 14H, CH2) 2.76 (s, 1H, OH), 2.4 (s, 3H, CCH3).

Synthesis of **12-Bromo-3,6,9,12-tetraoxadodecan-1-ol.** To a 500 mL 3-neck RB flask equipped with a stir bar and condenser was added LiBr (17 g, 0.2 mol) and dry acetone (200 mL). 12-(*p*-Tolylsulfonyl)-3,6,9,12-tetraoxadodecan-1-ol (8.0 g, 0.026 mol) was added and the mixture refluxed for 6 hr at 80 °C. The mixture was cooled to room temperature and stirred overnight. The solvent was removed in vacuo, and 150 mL of chloroform was added to the residue. The white precipitate was vacuum filtered and washed with chloroform. The chloroform layer was extracted 2X with water and dried over MgSO4. The solution was evaporated to yield a yellow oil. The product was purified via column chromatography using 10% MeOH in CHCl3 to give a yellow oil. (4.5 g, 73%). ^{1}H-NMR (300 MHz; CHCl3)**:** 3.81 (t, 2H, C*H*2OH), 3.73 (t, 2H, OC*H*2), 3.68 (t, 8H, OC*H*2), 3.61 (t, 2H, OC*H*2), 3.48 (t, 2H, CH2Br), 2.52 (s, 1H, O*H*).

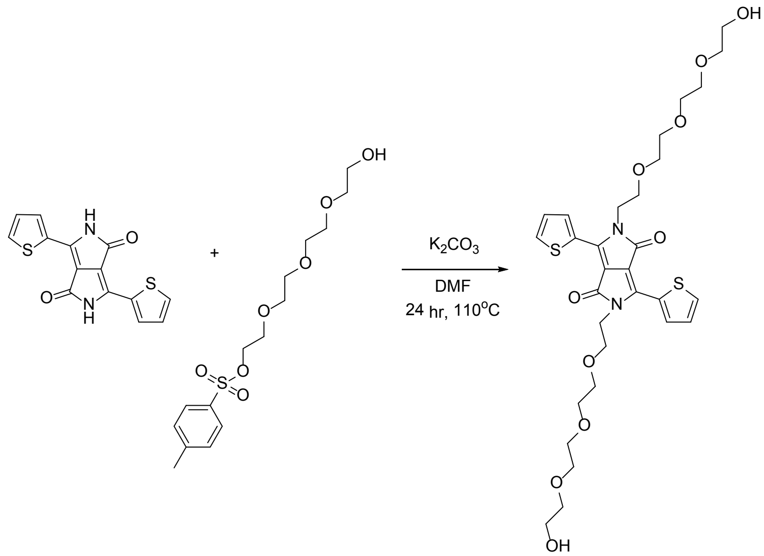
Synthesis of **FA-PEG-Br.** In a 100 mL flask equipped with a nitrogen inlet was added folic acid (1 g) and DMSO (25 mL). The mixture was stirred for 2 hr to dissolve the folic acid. 12-Bromo-3,6,9,12-tetraoxadodecan-1-ol (0.8 g) was added to the mixture along with DMAP (200 mg), pyridine (3 mL) and DCC (1.64g). The mixture was stirred overnight at RT. The DCU precipitate was vacuum filtered and washed with DMSO. The DMSO solution was added dropwise via funnel to a vigorously stirring diethyl ether solution. The precipitate was vacuum filtered and dried in air to yield a yellow solid.(3.2 g, %) ^{1}H-NMR (300 MHz; DMSO)**:** 11.4 (s, 1H), 8.61 (d, 2H), 8.0 (m, 2H), 7.36 (d, 2H), 6.96-6.68 (d, 2H), 6.55 (d, 2H), 4.47 (d, 2H),

*Synthesis of FA-functionalized 3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (**A2**).* The first step of the procedure outlined above for compound 1 will remain the same. For compound **2**, instead of adding 2-ethylhexylbromide, **FA-Br** will be added to give compound **4**. Compound **4** will undergo the same bromination procedure as outlined above for compound 3 to give **A2**.

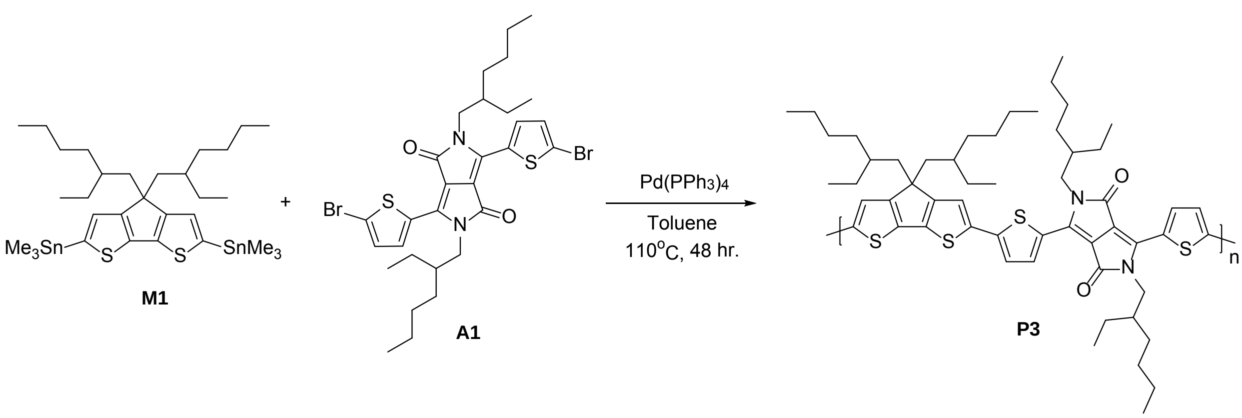


Scheme 2: The synthesis of FA-functionalized 3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione (**A2**)

***2,5-Di(3,6,9,12-tetraoxadodecan-1-ol)-3,6-dithiophen-2-ylpyrrolo[3,4-c]pyrrole-1,4-dione*.** Compound 1 (0.5 g) was added to DMF (15 mL) in a 2 neck 100 mL round bottom flask under nitrogen along with anhydrous potassium carbonate (2 g), TBAB (125 mg) and 12-(*p*-tolylsulfonyl)-3,6,9,12-tetraoxadodecan-1-ol (2 mL). The mixture was stirred at 120oC under nitrogen for 40 hr. The solution was cooled to room temperature and evacuated. The product was taken up in CHCl3 and extracted with water, 2N HCl and finally water. The combined organic extracts were dried over magnesium sulfate and evacuated to give a purple oil. The purple oil was subject to column chromatrography (8:2 to 6:4 CH2CL2:acetone) to give a red oil. (%). ^{1}H-NMR (300 MHz; CDCl3)**:** 8.75 (d, 2H), 7.65 (d, 2H), 7.26 (d, 2H), 4.28 (t, 4H), 3.82 (t, 2H), 3.71-3.46 (m, 28H).



Synthesis of poly[4,4-bis(2-ethylhexyl)cyclopenta[2,1-*b*;3,4-*b′*]dithiophene-2,6-diyl-*alt-2,5-Diethylhexyl-3,6-bis(thiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione*] (P3). 4,4-*Bis*(2-ethylhexyl)-2,6-*bis*(trimethyl-stan-nyl)-4H-cyclopenta-[2,1-b;3,4-b']dithiophene (728.3 mg) was added to a 250 mL 3 neck round bottom flask with *2,5-Diethylhexyl-3,6-bis(5-bromothiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione* (682.5 mg) and 40 mL of toluene. The solution was stirred and degassed for 15 minutes. $Pd(PPh_3)_4$ (100 mg) was added and the solution was further degassed for 15 min. The solution was heated to 120°C for 24 hours. Upon cooling to room temperature a viscous solution of blue/green polymer was observed in the reaction vessel. The polymer was precipitated in methanol and collected by vaccum filtration. The solid was then transferred to a Soxhlet thimble and subjected to extraction with MeOH (3 hrs), hexanes (6 hrs), and finally chloroform (6 hrs). The chloroform extract was evaporated almost to completion and methanol was added to precipitate the polymer, which was filtered and dried under vacuum for 24 hours (Yield 82 mg).



Scheme 3: The synthesis of poly[4,4-bis(2-ethylhexyl)cyclopenta[2,1-*b*;3,4-*b′*]dithiophene-2,6-diyl-*alt-2,5-Diethylhexyl-3,6-bis(thiophen-2-yl)pyrrolo[3,4-c]-pyrrole-1,4-dione*] **(P3).**

TRAINING OR FELLOWSHIP AWARDS: For training or fellowship awards, in addition to the elements outlined above, include a brief description of opportunities for training and professional development. Training activities may include, for example, courses or one-on-one work with a mentor. Professional development activities may include workshops, conferences, seminars, and study groups

I took translational approach to my research by collaborating with a physicist, Dr. David Carroll, as well as a surgical oncologist, Dr. John Stewart IV, on this project. Dr. David L. Carroll is the head of the Center for Nanotechnology and Molecular Materials at Wake Forest University. Dr. Carroll is a distinguished researcher, having published over 200 peer reviewed articles on nanotechnology. Dr. Carroll and I collaborated, during my Ph.D., on the synthesis and characterization of donor-acceptor conjugated polymers for organic solar cell applications. Through collaboration with Dr. Carroll, I have gained the necessary experience needed to become a leader in synthetic nanotechnology. I also chose Dr. John Stewart IV, M.D., of the Department of General Surgery, Section on Surgical Oncology as a collaborator on this project due to his extensive knowledge and training in treating metastatic colorectal cancer. Dr. Stewart's clinical interests are in general surgical oncology with a focus on peritoneal surface malignancies. He has assisted me through one on one meetings with discussions pertaining to animal models and surgical techniques needed to complete this project. Together my mentor, we have outlined the necessary training activities. My mentor and collaborators have all been available for questions, career development advice and help with experimental design methods. Some of the career development activities I have completed, so far, can be seen below.

Research Group Meetings and Mentor Meetings: I have met with my research mentor weekly and with my collaborators twice a month to discuss research directions as well as to evaluate my progress toward my ultimate goal of establishing a career as an independent investigator in cancer nanotechnology. I have continued to attend weekly research meetings in the Department of Plastic and Reconstructive Surgery and the Department of Surgical Sciences, where I am exposed to the clinical aspect of our research projects. I have presented three times since my fellowship started giving updates on my progress and answering questions from the group.

Intramural Seminar Series: I was able to attend weekly seminars and lectures in different departments and institutes at Wake Forest University Baptist Medical Center and Wake Forest University, such as the Translational Science Institute (TSI), School of Biomedical Engineering Sciences (SBES), the Comprehensive Cancer Center, the Departments of Chemistry and Cancer Biology and the Center for Nanotechnology and Molecular Materials. These seminars helped to expose me to inspiring research from different scientists and clinicians. This has allowed me to develop additional collaborations and to promote my individual research.

Society Memberships: I have continued to be involved in the American Chemical Society and the Materials Research Society, while pursuing new memberships in other professional organizations such as the Biomedical Engineering Society, Society for Thermal Medicine and American Society for Nanomedicine.

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